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DNA microarray-chip based diagnosis of Q-fever (*Coxiella burnetii*)

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INTRODUCTION

Coxiella burnetii is an obligate intracellular bacterium causing Q-fever, a zoonotic disease that is ubiquitous throughout the world with the exception of New Zealand. Cattle, goats, sheep and ticks are the primary reservoirs, but many other species, including fish, birds, rodents and cats, are known to become infected. Especially in small ruminants, infection can lead to abortion associated with exceptionally high amounts of *C. burnetii* in amniotic fluids and the placenta. Furthermore, *C. burnetii* may be excreted in milk, urine and faeces of infected animals. Human infections frequently follow contact with infected sheep, especially during lambing, or via the inhalation of dried tick faeces during shearing.

Most cases of human infection with *C. burnetii* are self-limiting, associated with fever, fatigue, headache and myalgia. Acute Q-fever is frequently accompanied by atypical pneumonia and/or hepatitis. Persistence of infection exceeding 6 months in duration is regarded as chronic Q-fever. Commonly, endocarditis is seen, but chronic hepatitis, osteomyelitis, septic arthritis or infection of aneurysm and vascular grafts can also occur. During pregnancy, Q-fever has been associated with premature birth, abortion and neonatal death.

Diagnosis mostly relies on serology, but, especially in the early clinical stages, PCR offers substantial benefits for the identification of *C. burnetii* infection [1].

In our study, we investigated the suitability of a new rapid 'low cost and density' (LCD) DNA microarray chip for the detection of *C. burnetii* (Coxiella 2.5). Besides the commonly used IS1111 genomic target (Genebank accession number: M80806), we coated a recently described genomic marker proposed to be diagnostic of acute Q-fever (acute disease antigen A (*adaA*), CBU_0952, GenBank ID AAO90475.1) on the chip [2]. The results were compared with those of both conventional, gel-based and a real-time LightCyclerHyprobe–PCR assay in terms of specificity and sensitivity. Clinical materials of both human and animal origin were also used for evaluation.

MATERIALS AND METHODS

We investigated 22 *C. burnetii* isolates from either humans or from animal species (Table 1). DNA was prepared from heat-inactivated cell culture material using the cell culture preparation-kit (MagNA Pure Compact; Roche, Mannheim, Germany). In addition, two human clinical samples and 50 samples from goats, sheep and cattle were assessed. DNA was prepared with a standard kit (DNA-Mini; Qiagen, Hilden, Germany). Specificity was assessed by using a DNA panel of 16 related bacteria. Sensitivity was determined by a dilution series of the IS1111 and *adaA* plasmid standards provided with the kit.

The LCD-Array Coxiella 2.5 Kit consists of a DNA microarray chip constructed from a transparent pre-structured polymer support slide (50 × 50 mm) containing eight identical hybridization fields spotted with immobilized IS1111 or *adaA* gene oligonucleotides (IS1111 probe 1, gATCgTAACgATgCgCAggC; IS1111 probe 2, gAACCATTggTATCggACgTTTATg; *adaA* probe 1, CTgAgCTgCgCCAgCgAg; *adaA* probe 2, AAgAATTAACgCTAATgCgATCTgC). All *Coxiella*-specific oligonucleotides were printed in duplicate. Three spots served as hybridization, staining and internal (lambda phage) inhibition controls. Visualization was achieved using a precipitation substrate (biotin–avidin complex) permitting easy interpretation by the naked eye. All primer solutions (IS1111-Mix F, 5′-ggTAAAgTgATCTACACgAgACgg; IS1111-Mix R, 5′-BIO-TCTTTAACAgCgCTTgAACgTC; adaA-Mix F, 5′-AATAgATTCgCTCTCTCAAgCCg; adaA-Mix R, 5′-BIO-ggTTTCTTCCCAAAgTCACCg; lambda-Mix F, 5′-ATgCCACgTAAgCgAAACA; lambda-Mix R, 5′-BIO-gCATAAACgAAgCAgTCgAgT), controls (IS1111 and *adaA* plasmid standards and lambda phage) are included in this ready-to-use kit. Following the PCR amplification step, material was simultaneously processed on the LCD array (hybridization, labelling and staining) and with both conventional and real-time LightCyclerHyprobe–PCR for confirmation (the latter techniques are described elsewhere [2,3]).

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No conflicts of interest declared.

Table 1. *Coxiella burnetii* isolates tested

Strains	Source/Origin	IS1111	*adaA*
Geier	Human, pneumonia, Romania	+	+
CS-Florian	Human, blood, Slovakia, 1954	+	+
Herzberg	Human, Greece	+	+
Balaceanu	Human, pneumonia, Romania	+	+
Brasov	Human, pneumonia, Romania	+	+
Henzerling	Human, blood, Italy, 1945	+	+
F-1	Human, endocarditis, France, 1992	+	–
CS-S1	Cow, Slovakia, 1970	+	+
CS-KL 1	Tick, Slovakia	+	+
CS-KL 5	Tick, Slovakia	+	+
Nine Mile	Tick, USA, 1938	+	+
Z2534/90	Goat, Austria, 1990	+	+
Hardthof/90	Cow, Germany, 1990	+	+
S-1	Sheep, Sweden, 1990	+	+
Z3749/92	Cow, Germany, 1992	+	+
Z104/94	Sheep, Germany, 1994	+	+
Z257/94	Cow, Germany, 1994	+	+
J-1	Cow, Japan	+	+
Frankfurt	Cow, Germany, 1982	+	+
München	Sheep, Germany, 1969	+	+
Priscilla	Goat, USA, 1980	+	–
Dugway	Rodents, USA, 1958	+	+

+, amplification of IS1111 or *adaA* gene sequences; –, no amplification.

RESULTS

All 22 *C. burnetii* isolates from animals and humans, regardless of whether they were from acute or chronic disease, yielded positive reactions with both probes in each PCR platform, with the exception of one human chronic disease-derived and one animal-derived isolate that failed to react with *adaA* regardless of the platform used (Table 1). *C. burnetii* DNA could be detected in different clinical matrices, including sputum, placental tissues, milk, faeces, and vaginal secretions (results not shown). The detection limit was 10 genomic copies per microlitre for IS1111 and 100 copies for *adaA* respectively for all three PCR methods. No detectable reactions were seen with DNA panels of 16 related bacteria, or 14 clinical samples from non-infected animal species or from uninoculated cell culture material. Thus, traditional and new PCR methods showed no difference according to sensitivity and specificity (100%).

CONCLUSION

This novel technique allows rapid (45 min with 15 min of 'hands-on' time only), sensitive, specific and economic detection of PCR-amplified products. Pure isolates and clinical material of human and veterinary origin could be successfully analysed. The LCD chip kit can be used in routine laboratories, as it does not require sophisticated equipment. Its low cost and simplicity make this diagnostic approach favourable for field or epidemiological studies in developing countries. In addition, we showed in this study that the *adaA* gene occurs in acute human Q-fever isolates. Nevertheless, further investigations of a larger collection, especially with chronic isolates, is recommended to confirm the value of this new acute disease marker.

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